Continental J. Medical Research 1: 13 – 23, 2007

DESIGN OF KETOCONAZOLE MICROSPHERES - FORMULATIVE APPROACHES

Nayyar Parvez[1], Suman Yadav[2],A.K.Bakhshi[3]
[1]Department of Pharmaceutics, Advanced Institute of Pharmacy, MD University Rohtak, Distt. Faridabad, Haryana, India. [2]Department of Chemistry, Advanced Institute of Technology, MD University Rohtak, Distt. Faridabad, Haryana, India. [3]Department of Chemistry, University of Delhi – 110007, India

ABSTARCT
Sustained release microspheres containing ketoconazole were prepared using 2^2 factorial design and were characterized for their release properties and micromeritic properties (intraquartile coefficient of skewness and kurtosis). The surface topography of the microspheres was investigated with scanning electron microscopy. In vitro drug release studies were performed using modified USP apparatus I in 0.1N HCl pH 1.20 for 2 h followed by phosphate buffer pH 7.20 as dissolution medium at 37°±0.5°C at 50 rpm for the next 6 h. Maximum drug release was observed with the KTZ microbead prepared with initial drug loading of 25%w/w. 69.62% of KTZ was released in 0.1 N HCl at 2 h and thereafter it sustained and approached 51.22% at the end of 8 h in phosphate buffer .The drug loading was in accordance with Baker Lonsdale model and the diffusion exponent value of 0.67 suggested non-fickian diffusion as the mechanism of drug release.

The objective of the study was to design diffusion controlled multiparticulate drug delivery system of ketoconazole in order to sustain the delivery of the drug and thereby reduce the gastrointestinal disturbances and dose related adverse effects like hepatic dysfunction and allergic reactions as observed with conventional oral dosage form of ketoconazole.

KEYWORDS: Ketoconazole drug loading Baker Lonsdale model, Non fickian release.

INTRODUCTION

Fungal infections are common in human beings, which are either topical or severe systemic infection. Invasive fungal infections are being identified with an ever-increasing frequency in premature infants, immunocompromised hosts, patients receiving immunosuppressive agents, and in those with AIDS (Sweetman, 2002). The prevention and treatment of invasive fungal infection have been improved over the last two decades by introduction of newer antifungal agents such as ketoconazole. It is effective against candida aspergillus (Hoban *et al*, 2001) and unusual organism of fusarium(Marina *et al*, 1992) and pseudallescheria boyddii. It is also found to be effective in several systemic and topical fungal infection especially candidiosis (Datry *et al*, 2001) and cryptococcus (Kronfeld *et al*, 1991) associated with immuno suppression as in AIDS or cancer therapy.

The present study aims to develop and evaluate sustained release microspheres (Robinson and Lee, 1986) of the antifungal drug, ketoconazole. The oral absorption of ketoconazole is facilitated by gastric acidity (Vander Meer *et al*, 1980) because it is more soluble at lower pH (Zhou, 2005) and hence it was thought to prepare a multiple unit improved oral dosage form which could over come the problem associated with conventional tablets(Gupta and

Robinson 1992). Short biological half-life and low molecular weight (Martin, 1965) favored pharmacokinetic rationale for development of a sustained release dosage form.

The ketoconazole microspheres for controlled delivery of the drug by solvent evaporation (Lachman *et al*.,1991) method using dichloromethane, one of the class II solvents (ICH GUIDELINES Manual) proposed in ICH guidelines (Q3C) to be used in the pharmaceutical industry because of its low toxic potential, as the coacervating agent to reduce residual solvent.

MATERIALS AND METHODS

Materials

Ketoconazole was obtained as gift sample from Torrent pharmaceuticals Ltd (Indrad) Gujarat, India. Ethyl cellulose and methylcellulose were purchased from S.D. fine chemicals Ltd, Mumbai, India, Dichloromethane was procured from Qualikems fine chemicals Pvt. Ltd., New Delhi, India. All glass distilled water was used throughout the study.

Preliminary trials for optimization of microspheres without drug

Microspheres were prepared by solvent evaporation method (Dubernet *et al*., 1991) using ethyl cellulose (EC) and methylcellulose (MC). The ratio of the polymers used were optimized using 2^2 factorial design. (Table 1). Ethyl cellulose (2gm) was dissolved in 20 ml of methylene chloride (Dhanaraju *et al*., 2003). The polymer phase was then added to 250 ml of 0.25% w/v methylcellulose aqueous solution (over night dispersion). Agitation was maintained at 350 rpm until complete evaporation of methylene chloride. Microspheres were then collected, washed three times with distilled water, filtered and stored under reduced pressure, overnight in a desiccator.

Additionally, optimization of the process was done by selecting suitable stirring element i.e. magnetic stirrer vs mechanical stirrer in order to improve the shape and yield of microspheres. Paired t- test was applied for final selection of the stirring element based on 95% confidence interval (Bolton 1990).

EVALUATION OF DRUG FREE MICROSPHERES

Micromeritic Studies

The particle size and particle size distribution of A_1- A_4 microspheres were measured using optical compound microscope by spreading minute quantities of microspheres on a clean glass slide. The mean diameter of 500-600 microspheres were measured and recorded (Bayomi *et al.*, 1994). The data was used for the calculations of various micromeritic parameters and was subjected to statistical analysis.

Selection of optimized microbeads

Optimized microspheres A_4 without drug were selected on the basis of uniformity of shape, average diameter, percentage yield, and other micromeritic parameters. Further, the selection of stirring element was based on percentage yield and reduction in standard deviation of average diameter and was validated by t- test. The selected process was used for preparation of drug-loaded microspheres.

PREPARATION OF KETOCONAZOLE MICROSPHERES

Microspheres of KTZ were prepared using solvent evaporation method. Ethyl cellulose (2gm) was dissolved in 20 ml of methylene chloride and KTZ was added to this solution, in varying amounts, corresponding to theoretical initial loading range from 5 to 40%w/w (F_1- F_7), Table 2.

The polymer phase was then added to 250 ml of 0.25% w/v methylcellulose aqueous solution (over night dispersion). Agitation was maintained at 350 rpm until complete evaporation of methylene chloride. Microspheres were then collected, washed three times with distilled water, filtered and stored under reduced pressure, overnight in a desiccator to ensure complete removal of residual solvent. At the end of 15 minutes residual ketoconazole microspheres were again subjected to 0.1N HCl and absorbance of filtered extract was determined. It was found to be negligible with negative absorbance to the tune of 0.001 and TLC of the sample did not show any spot corresponding to KTZ.

EVALUATION OF KETOCONAZOLE MICROSPHERES

Drug entrapment efficiency

Ketoconazole Microspheres (10mg) were dissolved in 0.1 N HCl and it was shaken in a vortex mixture for 15 minutes and centrifuged at 350 rpm and the formulations F_1-F_5 were subjected for further studies.

Micromeritic studies

Microspheres were observed by optical microscopy for particle size and its distribution using compound microscope under 10X magnification. The particle size and its distribution, of F_1-F_5 formulations were measured using the microscope. The diameter of 500-600 microspheres were measured and the data obtained were plotted on log-probability scale and used for calculations of average bead size, standard deviation, IQCS (intra quartile coefficient of skewness), coefficient of kurtosis.

In vitro dissolution study

Microspheres equivalent to 200 mg of KTZ were filled in transparent, zero size hard gelatin capsule were evaluated for in vitro drug release study. The study of F_1-F_5 formulations were carried out in accordance with USPXXIV Type I rotating basket apparatus (Hicon, New Delhi) using 900 ml of 0.1 N HCl for 2 hrs followed by dissolution in phosphate buffer for 6 hrs at 50 rpm (USP NF 2004) A muslin cloth (200#) was tied over the basket to prevent the slippage of microspheres from the basket (Gohel and Amin 1999).. The filled hard gelatin capsule was placed in the basket and 5 ml samples were withdrawn at regular time intervals replacing with an equal amount of fresh dissolution medium (37±0.5º C) immediately after withdrawl of test samples. The samples were filtered, diluted suitably and analyzed spectrophotometrically (Pharma Spec1700, Shimadzu Japan) at 269.0 nm for samples in 0.1N HCl and 280.5 nm for samples in phosphate buffer. The percentage drug dissolved at different time intervals was calculated. The study was performed in triplicate for each batch and the data obtained was plotted against time.

Model fitting

Data obtained from in vitro release studies was fitted to various kinetic equations to find out the mechanism of KTZ release from ethyl cellulose microspheres. The kinetic models used were zero order (Baveja et al., 1987), first order (Wagner, 1969), Higuchi (Higuchi et al., 1963), Peppas model (Korsemeyer et al., 1983). The following plots were made: Q_t vs. t (zero order kinetic models); log (Q_o-Q_t) vs t (first order kinetic model); Q_t vs square root of t (Higuchi model). Where Q_t is the amount of drug released at time t and Q_o is the initial amount of drug present in the microspheres, $Q = kt^n$ (peppas model) where Q is the amount of drug release; t is time; k is the constant incorporating structural and geometrical characteristic of the release device and n is the release exponent indicative of the mechanism of release. Plots were subjected to regression analysis to find out the regression coefficient and hence the order of release.

SELECTION OF OPTIMIZED FORMULATION OF KTZ MICROSPHERES

On the basis of maximum drug entrapment efficiency, an IQCS value approaching zero and best-controlled drug release, the formulation F_5 was selected as the optimized formulation and was subjected to further studies.

SCANNING ELECTRON MICROSCOPY FOR SURFACE TOPOGRAPHY

The surface topography of F_5 formulation of microspheres was investigated with scanning electron microscopy. Microspheres samples were mounted on to stubs using double sided adhesive tape and vacuum coated with gold film (10A°) by a polaron sputter coater E5100 and analyzed by a SEM (JEOL, JSM-T220, Japan).

STABILITY STUDIES

The prepared microspheres (20 mg) were placed in sealed, clear glass vials and stored at ambient humidity conditions at room temperature (25°±2°C), oven temperature (45±2°C) and in refrigerator (5-8°C) for a period of 60 days (Guo, 1994). The samples were assayed for drug content and evaluated for physical stability at regular interval of one month. The samples withdrawn were also subjected to thin layered chromatography.

RESULTS AND DISCUSSION

PRELIMINARY TRIALS FOR OPTIMIZATION OF MICROSPHERES WITHOUT DRUG

Preparation

Microspheres were prepared by solvent evaporation method using ethyl cellulose and methylcellulose. The solvent evaporation method is a popular microencapsulation technique for the preparation of drug containing matrix particles from water insoluble polymer for controlled release of drug. The control depends upon the nature of polymers and the concentration of polymers selected.

Therefore, concentration of polymers to be used were optimized by using 2^2 factorial design .On the basis of the design, microspheres without drug were prepared (A_1-A_4) using ethyl cellulose (5 and 10% by weight) and methyl cellulose (0.125% and 0.250% by weight). It was observed that high levels of both ethyl cellulose and methylcellulose yielded spherical shape microspheres with an average diameter of 283.41μm. Thus, for further studies A_4 with high levels of EC and MC were selected.

Further, the process was also optimized in terms of suitable stirring element i.e. mechanical stirrer and magnetic stirrer using A_4, in an attempt to improve the shape and yield of microspheres. It was found that mechanical stirrer was superior in comparison to magnetic stirrer as it resulted in increased yields and decrease in the average diameter both favourable for process of preparation (Table 3). The superiority of mechanical stirrer method was further accenuated on application of t – test, using graph pad software. It was found that there was a significant difference (P> 0.5%) between the methods used, at 95 % confidence interval.

EVALUATION

Micromeritic studies

The particle size and particle size distribution of microspheres A_1- A_4 were measured using optical compound microscope. The data obtained was plotted on log probability scale and the average mean diameter along with other statistical micromeritic parameters are listed in table 4.

A_4 was selected as the best formulation for drug loading as it resulted in an average diameter of 283.41 μm with least value of standard deviation 1.58 and an IQCS value of – 0.121 which approximates to zero thus suggesting near symmetrical distribution between the quartile points. To quantify the degree of symmetry of particle size distribution a property known as kurtosis can also be determined. The symmetry of distribution is based on the comparison of the height and thickness of the tail and sharpness of peak with those of normal distribution. A negative value for coefficient of kurtosis suggests platykurtic distribution i.e. a thin tailed and blunt peaked curves of particle size distribution. The A_4 formulation also exhibits platykurtic distribution suggesting higher frequency of small sized microspheres. This means that a higher percentage of microspheres lie in the range of 281.83 - 283.41μm.

Preparation of ketoconazole microspheres

KTZ microspheres were prepared using solvent evaporation method with the aid of mechanical stirrer as stirring element. The selected A_4 formulation was subjected to initial loading range from 5 – 40 %w/w and the prepared microspheres were evaluated for the below detailed parameters.

EVALUATION OF KETOCONAZOLE MICROSPHERES

Drug entrapment efficiency

The drug entrapment efficiency of F_1-F_7 shown in (Fig.1.) clearly indicates that with increase in initial drug loading from 5 – 25 % by weight the drug entrapment efficiency increased linearly thereafter a sharp decline was observed in entrapment efficiency with drug loading values of 30 –40% (Jain *et al*., 1998). In vitro drug release study of F_5 – F_7 formulations (25%, 30% and 40% by weight of drug loading) was carried out in 0.1 N HCl for 2 hrs followed by dissolution in phosphate buffer pH 7.20 for a period of 6 hrs. The experiment revealed that, the drug release (Fig 2) follows Baker – Lonsdale model (Costa and Lobo 2001). According to this model, for a dispersed drug, the fraction of drug released decreases with an increase in the initial drug loading whereas, for the dissolved drug, the fraction released at any time is independent of initial loading. This model was developed by Baker and Lonsdale from Higuchi model and describes the drug-controlled release from a spherical matrix. One-way ANOVA followed by scheffs pairs wise comparison did not reveal any significant difference. For the development of formulation F_1-F_5 were subjected to elaborate evaluation parameters for selection of optimized KTZ microspheres.

Micromeritic studies

All the formulations of KTZ microspheres F_1 – F_5 (Table 5) were spherical shaped but the difference in micromeritic properties helped in selection of the best formulation. The F_5 formulation with an average mean particle diameter of 353.6µm with a standard deviation of 1.43 was identified as superior formulation with maximum drug content of 4.854 mg per 10 mg of the microspheres. Particle size distribution when summarized using statistical methods exhibited an IQCS value of – 0.023 which is quite close to zero. If the IQCS value is zero the size distribution is practically symmetrical between the quartile points.

The value –0.023 being close to zero indicates an almost symmetrical distribution and a low value of standard deviation indicates uniformity in beads size. Uniformity is essentially important for controlled release of the drug, which will also help in maintaining uniform therapeutic concentration for the desired time period thus avoiding fluctuation in drug levels. Platykurtic distribution further quantifies the degree of symmetry of the microspheres and indicates higher percentage of finer microspheres desirable for multiple unit controlled release dosage form (Mahrouk *et al*., 1993).

In vitro dissolution study

Plots of % drug release vs time profile exhibited biphasic release behaviour of ketoconazole from microspheres (Fig. 3). Initially, a burst effect with in 2 hrs was observed predominantly in F_4 and F_5 formulations, thereafter a period of slow release followed till 8 hrs. This biphasic behavior was not significant with formulations with low initial loadings i.e. F_1- F_3. This suggests that the release behaviour is dependent on the amount of the drug present in microbeads (Muthushamy *et al.,* 2004, Perugini *et al*., 2000).

The basic nature of the drug ketoconazole permits solubility of drug in the acidic media used for the test. This, complemented with rapid drug diffusion through dissolution media-filled pores and channels explains the burst effect of drug release in F_4 and F_5 formulations. The slower release phase can be attributed to higher partitioning of the drug to sphere matrix as compared to the dissolution medium (Pothal *et al*., 2004). This is again due to basic nature of drug, which does not support its solubility in the tested basic medium. Various other factors that can affect the drug release from the microspheres include the bead size and its morphology, physical state of the drug in the polymer and the type of polymer (Mishra *et al*., 2003).

Model fitting

In order to obtain meaningful information for the release models, the drug release profiles were fitted to various kinetic models. Table 6 summarizes the correlation coefficient for the different release kinetic models of KTZ beads. Models with higher correlation coefficient were judged to be a more appropriate model for the dissolution data. The dissolution fitted Higuchi and Peppas model with r^2 values of 0.9216 and 0.9134 respectively suggesting diffusion as the mechanism of drug release. The diffusion exponent value (n) was found to be 0.67, which is greater than 0.45 thus confirming the non-fickian release.

SCANNING ELECTRON MICROSCOPY FOR SURFACE TOPOGRAPHY

The surface topography of microspheres was investigated with SEM. It is vivid from SEM photomicrograph (Fig. 4) that the beads were uniform, spherical with rough surfaces and an average diameter of 363.33µm ± 1.21. The rough surfaces of the spheres can be attributed to rapid solvent diffusion during the preparation of microspheres (Polk *et al*., 1994). This surface morphology is also supported by literature, which reports that microspheres with rough surfaces are produced when solvent evaporation technique is used (Puglisi *et al.,* 1992).

CONCLUSION

Thus the prepared KTZ microspheres exhibited sustained release behaviour, which was pH dependent with the release process being diffusion controlled without matrix erosion. The formulated microspheres were stable and could be further modified for the development of pH independent oral controlled release microbead by addition of enteric polymer (anionic poly electrolytes). The enteric polymer contributes as matrix base in acidic media and increased the pores for drug release caused by dissolution of enteric polymer in neutral and basic media thus resulting in pH independent release in the whole gastro intestinal tract.

Table 1. Factorial design for the optimization of the polymers.

Factor combination	Ethvl cellulose	**Methylcellulose**
A_1	-	-
A_2	+	-
A_3	-	+
A_4	+	+

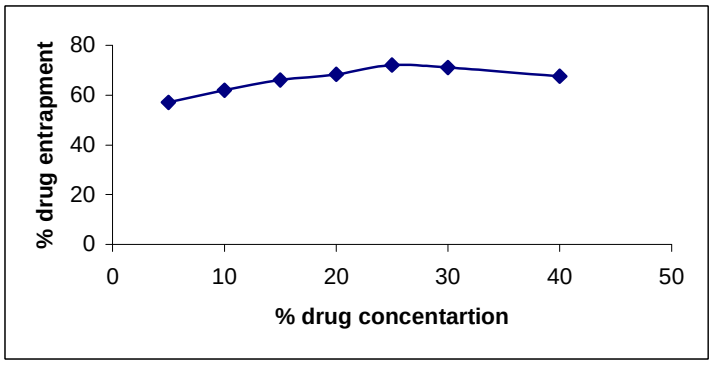
Key (+) - High level, (-) - Low level

Table 2. Initial drug loading and their corresponding formulation codes

S.NO.	INITIAL LOADING OF DRUG (% w/w)	FORMULATION CODE
1	5	F1
2	10	F2
3	15	F3
4	20	F4
5	25	F5
6	30	F6
7	40	F7

Table 3. Superiority of the mechanical stirrer in comparison to the magnetic stirrer.

S.No.	Factor	Response	Percentage response
1	Average diameter	Decreased	0.138*
2	Percentage Yield	Increased	2.4*
3	Standard deviation	Same	No change



($p < 0.5$)

Fig. 1. The effect of variable drug concentration on the percentage entrapment of ketoconazole in microspheres.

Table 4. Micromeritic parameters of the drug free microspheres.

Facto	Response					
	Shape	Averag e	Percentag e yield	IQCS	Standard Deviatio	Kurtosis
A_1	Irregula r	191.82	41.67	0.311	1.98	Leptokurtic
A_2	Spheric al	244.80	60.49	- 0.0769	1.61	Platykurtic
A_3	Spheric al	150.22	42.67	0.125	2.83	Leptokurtic
A_4	Spheric al	283.41	60.80	-0.121	1.58	platykurtic

Table 5. Physicochemical and Micromeritic data of formulations F_1 to F_5 of ketoconazole microspheres.

Factor	Shape	Average diameter	% Yield	Drug content	IQCS	Standar d	Kurtosis
F_1	Spherical	203	71.55	2.199	0.152	2.12	Leptokurtic
F_2	Spherical	321.0	77.03	2.323	0.0342	1.68	Leptokurtic
F_3	Spherical	252.2	79.08	3.651	0.0322	1.59	Leptokurtic
F_4	Spherical	333.6	82.30	3.775	-0.028	1.35	Platykurtic
F_5	Spherical	353.6	84.29	4.854	-0.023	1.43	Platykurtic

Table 5

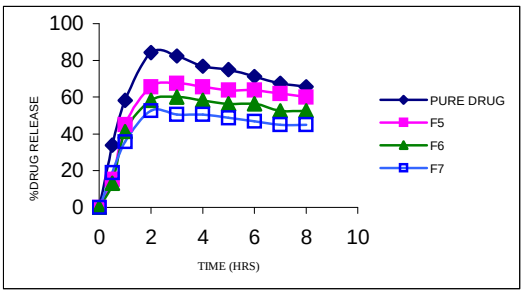


Fig. 2. Drug release profiles of F5 to F7 in 0.1 N HCl for 2 hrs followed by phosphate buffer pH 7.20 for 6 hrs justifying Baker Lonsdale model.

Table 6. Kinetic model treatment of dissolution profiles of formulations $F_1 – F_5$.

S.No.	Time (months)	Drug content under variable storage conditions		
		Room temperature (25°±2°C)	Oven temperature (45±2°C)	Refrigerator (5-8°C)
1	0	4.854	4.854	4.854
2	1	4.801	4.813	4.639
3	2 p	4.799	4.819	4.566

Table 7. Stability data of KTZ microspheres stored under variable conditions

Formulation	Zero order r^2	First order r^2	Higuchi plot r^2	Peppas plot r^2
F_1	0.8575	0.8618	0.9183	0.9045
F_2	0.7904	0.7850	0.9085	0.8876
F_3	0.8927	0.8783	0.8976	0.8962
F_4	0.8511	0.8293	0.9116	0.9025
F_5	0.8711	0.7942	0.9216	0.9134

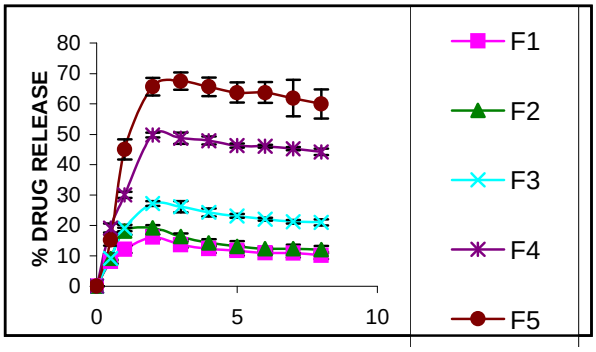
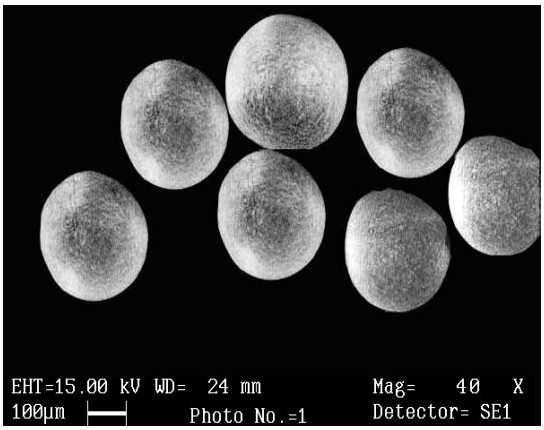
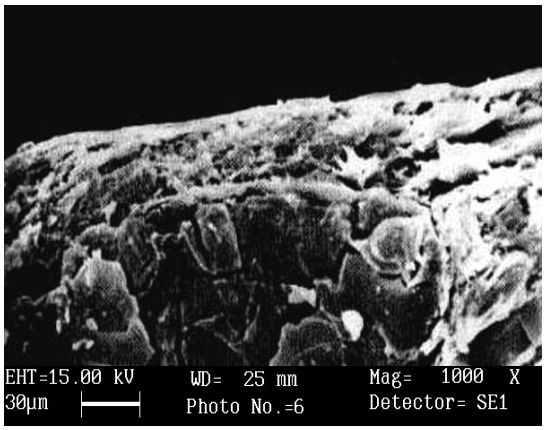


Fig. 3. Comparative in vitro study of all the formulations of KTZ ($F_1 – F_5$) with respect to pure drug in 0.1 N HCl for 2 hr followed by phosphate buffer pH 7.20 for 6 hr.





. b.

Fig. 4. Scanning electron micrographs of (a) KTZ microspheres and its (b) surface.

REFERENCES

Baveja SK, Ranga Rao KV, Padmalatha K (1987). Zero order release hydrophilic matrix tablet of beta adrenergic blockers Int J Pharm 39: 39-45.

Bayomi AM, El-Sayed MY (1994). Casein microbeads as a controlled parenteral drug delivery system. Drug Dev Ind Pharm 20: 2607-2614.

Bolton S (1990). Pharmaceutical statistics practical and clinical application, Marcel Dekker, New York, 2nd edn, revised and expanded, 44, p.222.

Costa P, Lobo JM (2001). Modelling and comparison of dissolution profiles. Eur J Pharm Sci 13:123-129.

Datry A, Thellier M, Traore B, Alfa Cisse O, Danis M (2001). Antifungal drugs in the treatment of systemic candidiosis: susceptibility to antifungal drugs, point on drug resistance, pharmacological data. Annales Francaises d'Anesthesie et de Reanimation 20: 389-393.

Dhanaraju MD, Bhaskar K, Vamsadhara C (2003). A multiple emulsion method to entrap hydrophilic compound into ethylcellulose microspheres. Indian Drugs 40:99-104.

Dubernet C, Rouland JC and Benoit JP (1991). Ibuprofen loaded ethylcellulose microspheres: analysis of the matrix structure by thermal analysis. J Pharm Sci 80:1029-1035.

Gohel MC, Amin FA (1999). Studies in the preparation of diclofenac sodium microspheres by emulsion solvent evaporation technique using response surface analysis. Indian J Pharm Sci 61: 48-55.

Guo JH (1994). Preparation methods of biodegradable microspheres of bovine serum albumin loading efficiency and release profiles. Drug Dev Ind Pharm 20:2535-2545.

Gupta PK, Robinson JR (1992). Oral controlled drug delivery, Treatise on controlled drug delivery, Marcel dekker,.286-290.

Higuchi WI, Fawzi N, Katdare T (1963). Model for the dissolution of calcium hydroxypatite powder. J Phys Chem 96: 861-869.

Hoban DJ, Weshnoweski B, Bellyou T, Palatnick L, Zhanel GG (2001). Azole Resistance in Candida, Aspergillus & Penicillium species: Results of a Three Year Canadian National Surveillance Study. Abstr Intersci Conf Antimicrob Agents Chemother Intersci Conf Antimicrob Agents Chemother 41:16-19.

ICH Harmonised Tripartite Guideline Recommended for Adoption at Step 4 of the ICH Process on 12 September 2002 by the ICH Steering Committee.

Jain R, Navnit HS, Waseem M, Rhodes TC (1998). Controlled drug delivery by biodegradable poly (ester) devices: different preparative approaches. Drug Dev Ind Pharm 24:703-712.

Korsemeyer RW, Gurny R, Doelker E Peppas NA (1983). Mechanism of solute release from porous hydrophilic polymers. Int J Pharm 15: 25-35.

Kronfeld M, Sprinz E, Zimmer P (1991). Ketoconazole as a prophylactic agent against cryptococcal meningitis. Int Conf AIDS 258:16-21.

Lachman L, Liberman HA and Kaing JL (1991).The theory and practice of industrial pharmacy. 4th edition,412-414.
Mahrouk GM, Al-Meshal MA, Mahrous GM (1993). Preparation and evaluation of sustained release indomethacine nonpareil seeds. Drug Dev Ind Pharm 19:1903-1911.

Martin BK, (1965). Potential effects of the plasma Proteins on drug distribution. Nature, 207:274-278.

Marina L, Avigdor S, Ronni W, Edna B, Sarah B, (1992). Systemic ketoconazole treatment for fusarium leg ulcers. International Journal of Dermatology 31: 511-512.

Mishra B, Jayanth P, Sankar C (2003). Development of chitosan-alginate microcapsules for colon specific delivery of metronidazole. Indian Drugs 40:695- 699.

Muthushamy K, Shibi KP, Ravi TK (2004). Preparation and evaluation of albumin-chitosan microsphere containing theophylline Ind J Pharm Sci 65:245-249.

Perugini P, Genta I, Pavanetto F, Baruffini A (2000). Study on glycolic acid delivery by liposomes and microspheres. Int J Pharm 196:51-61.

Pothal RK, Sahoo SK, Chatterjee S, Barik BB (2004). Preparation and evaluation of mucoadhesive microcapsules of theophylline. The Ind Pharm 12:74-78.

Polk A, Amsden B, Yao de K, Peng T, Goosen MF (1994). Controlled release of albumin from chitosan-alginate microcapsules. J Pharm Sci 83:178-188.

Puglisi G, Giammona G, Santagati NA, Carlisi B, Villari A (1992). Preparation and biological evaluation of ethylcellulose microspheres containing tolmetin Drug Dev Ind Pharm 18:939-945.

Robinson JR and Lee VH (1986). Controlled drug delivery fundamentals and concepts, Marcel Dekker, New York, 2nd Edition revised and expanded, 29:5-20.

Sweetman SC (2002). Matrindale the complete drug reference 33rd Edition, 389-391.

United States Pharmacopoeia – National Formulary, Rockville, Maryland, 2004

Vander Meer JWM, Keuning JJ, Scheijgrond HW, Heykants J, Van Cutsem J, Brugmans J(1980). The influence of gastric acidity on the bioavailability of ketoconazole. J. Antimicrob. Chemother 6: 552- 554.

Wagner JG (1969). Interpretation of percent dissolved time plotsderived from in vitro testing of conventional tablets. J Pharm Sci 58: 1253-1260.

Zhou R (2005). pH-dependent dissolution in vitro and absorption in vivo of weakly basic drugs: development of a canine model. Pharm Res. 22:188-92.

Received for Publication: 11/10/2007
Accepted for Publication: 13/11/2007

Corresponding Author:
Suman Yadav
[2]Department of Chemistry, Advanced Institute of Technology, MD University Rohtak, Distt. Faridabad, Haryana, India. Email: drsumanyadav2007@rediffmail.com,